International Journal of Pharmaceutics, 26 (1985) 175–189
Elsevier

IJP 00882

Cutaneous biotransformation as a parameter in the modulation of the activity of topical corticosteroids

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(Received May 1st, 1985)
(Modified version received May 20th, 1985)
(Accepted May 25th, 1985)

Key words: skin enzymes – skin esterases – steroid activity – betamethasone-17-valerate – betamethasone esters – steroid biotransformation

Summary

The hydrolysis of betamethasone-17- and -21-valerates by hepatic and cutaneous esterases is studied with a view to explaining their different activity profiles. It is shown that the 17-ester is resistant to both esterases while the 21-ester is rapidly hydrolyzed to the free steroid alcohol. Earlier reports on the cutaneous enzymic transformation of the 17-ester fail to account for the spontaneous isomerization of the ester to the 21-ester and are therefore misleading. The resistance of the 17-ester to enzymic hydrolysis may also lead to a more pronounced reservoir effect and hence toxicity following application to the skin. This work provides useful information for the design of topically active steroids.

Introduction

The skin is now known to possess a range of metabolic activities (Ando et al., 1977; Bundgaard et al., 1983; Malkinson et al., 1959; Hsia and Hao, 1967; Oertel and Treiber, 1969; Pugliese, 1978). It may also serve as a reservoir for both endogenous and exogenous steroids (Lewbart and Mattox, 1959; Malkinson and

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Ferguson, 1955; Vickers, 1963; Wester et al., 1980). Abnormalities in cutaneous handling of endogenous steroids are thought to contribute to a range of clinical conditions including inflammation, acne, hirsutism and testicular feminization syndrome (Fauci et al., 1977; Hong and Levine, 1976; Kutten and Mauvais-Jarvis, 1981; Marks, 1977).

Cutaneous enzymic activities identified so far include esterase (Maruyama et al., 1980; Tauber and Toda, 1976), reductase (Takayasu et al., 1980), dehydrogenase (Hsia and Hao, 1967), oxidase (Greaves, 1971) and decarboxylase (Lesiewicz and Goldsmith, 1980; Murray et al., 1980). A good understanding of how such enzymes modulate the activity of topically applied drugs is therefore useful in the design of such drugs and their delivery systems particularly in relation to identifying the appropriate rate-limiting step. In this study, the susceptibility of topical steroid esters to esterases were chosen for study.

Esterification has been shown to be one of the most rewarding methods for modulating the activity and transport properties (Behl et al., 1984; Elias et al., 1981) of topical corticosteroids. Hydrocortisone acetate, for example is about 10 times as active as hydrocortisone in the standard vasoconstrictor assay for potency while hydrocortisone-17-butyrate is as effective as fluocinolone acetonide (McKenzie and Stoughton, 1962). With betamethasone valerates, the 17-ester is about 15 times more active than the 21-ester (McKenzie and Atkinson, 1964). Topical steroids and in particular the betamethasones therefore provided the required model compounds for this study.

Materials and Methods

Assay of betamethasone and its esters

A reversed-phase high-performance liquid chromatographic (HPLC) system was used for assaying betamethasone and its esters. The equipment used was as previously described (Yip et al., 1983). The mobile phase consisting of 55% acetonitrile in water was delivered at 1.2 $ml \cdot min^{-1}$ and the separation was on a 10 cm × 4.6 mm i.d. Hypersil-5 ODS (Shandon, U.K) column. Peak detection was at 250 nm and at sensitivity settings ranging from 0.04 to 0.32 AUFS.

Tris buffer solution

Each litre of pH 8.09 buffer solution contained 6.05 g of tris(hydroxymethyl)aminomethane and 279 ml of 0.1 N hydrochloric acid solution in water.

Tris buffer concentrated

Each litre of pH 8.25 solution contained 9.692 g of tris(hydroxymethyl)aminomethane and 446.4 ml of 0.1 N hydrochloric acid solution.

Acidified acetonitrile solution

This was prepared to contain 50% acetonitrile in 0.024 N aqueous hydrochloric acid solution.

Internal standard

Hydrocortisone-17-butyrate dissolved in acidified acetonitrile solution was used as internal standard for assaying betamethasone and its esters. The concentration used ranged from 0.012 to 0.06 $mg \cdot ml^{-1}$.

Hog liver esterase

The hog liver esterase (Sigma Chemicals) used in the metabolic studies was a commercially available purified carboxylic ester hydrolase obtained from hog liver and suspended in 3.2 M ammonium sulphate solution adjusted to pH 8. The claimed activity was that 1 unit hydrolyzed 1 μl of ethyl butyrate per minute at pH 8 and 25°C. Each mg of protein was equivalent to 120 units and each ml of solution contained 8 mg of protein. Where the enzyme solution was diluted prior to use, 3.2 M ammonium sulphate solution was used as diluent.

Cutaneous esterase

Cutaneous esterase was obtained from mouse skin. The whole skins of freshly killed mice were mechanically depilated and 3–4 g cut into tiny pieces and suspended in 20 ml of iced Krebs-Ringer solution of pH 7.51. The mixture was twice homogenized by passing through a french press (Aminco) precooled to − 4°C before use in order to minimize heat-induced denaturation of the cutaneous enzymes during processing. The homogenates were diluted with cooled buffer at about 4°C and centrifuged at 12,000 rpm at 4°C for 1 h. The supernatant was filtered through a 1.2 μl Millipore-MF membrane filter and diluted to 200 ml with buffer and stored at − 15°C until used.

Kreb's-Ringer solution

This pH 7.51 aqueous buffer was made up from 6.9 g sodium chloride, 0.35 g potassium chloride, 0.16 g potassium dihydrogen phosphate, 1.8 g sodium bicarbonate and 1.2 ml of 1 M calcium chloride solution per litre of solution. All the chemicals used were of Analar grade and obtained from British Drug Houses.

NADPH-generating solution

For studying the effect of the co-enzyme NADPH on the activity and stability of the esterases in the mouse skin homogenates, an NADPH-generating solution was prepared to contain 15 mg NADP and 50 μl of glucose-6-phosphate in 25 ml of Krebs solution.

Test solutions

Except where indicated, all the non-enzymic hydrolyses were carried out at 37°C in solutions containing 20% propylene glycol and 80% pH 8.09 Tris buffer solution. The final aqueous glycol buffer had a pH of 8.14. The propylene glycol was necessary because of the poor solubility of the steroid and of its esters. Prior solution in preheated propylene glycol eliminated the decomposition observed during dissolution when the steroids were dissolved directly in the buffer.

Enzymic hydrolysis

For the enzymic hydrolyses, 0.1 ml of enzyme solution was added to 50 ml of the steroid solution and thoroughly mixed. 1-ml aliquots were withdrawn at appropriate intervals and 1 ml of interval standard solution added. The acidified acetonitrile solution used for the internal standard quenched the reaction and enabled assay of residual steroid by HPLC. These acidified solutions were stable for at least 1 week at 4°C.

During the course of the mouse skin studies it was observed that a few of the solutions made up with Krebs solution showed pH changes of greater than 0.1 unit under the conditions of the study. These runs were discarded. To overcome this problem in the later studies comparing the enzymic transformation of the two betamethasone esters, Tris buffer was used instead of the Kreb's solution. The same buffer system was, however, used whenever comparisons were made.

Results and Discussion

Several factors can account for the differences in activity of the betamethasone esters: (a) the 17-ester may possess stereochemical features which are better than the 21-isomer for eliciting the desired effect. This is particularly important since recent work has shown evidence for the presence of steroid receptors in the skin (Epstein and Munderloh, 1971; Epstein and Bonifas, 1982; Hughes and Yardley, 1982); (b) the 17-ester may penetrate the skin better; (c) the 17-ester may be less susceptible to metabolic inactivation in the skin and in this context, cutaneous esterase activity may be of particular relevance; and (d) the 17-ester may be chemically more stable in the skin. In practical terms, this would mean that the 21-ester is converted to the less active betamethasone alcohol more rapidly than the 17-ester during release from the formulation.

Several authors have reported on the susceptibility of steroid esters to esterases from various tissues obtained from both man and animals. Rawlins et al. (1979), for example, claimed that betamethasone-17-valerate was susceptible to hydrolysis by human skin. The radiochemical method of assay used was, however, not suitable for discriminating between the 17- and 21-esters and no correction was made for spontaneous non-enzymic isomerization which has been reported on several occasions (e.g Bundgaard and Hansen, 1981; Gardi et al., 1963; Yip and Li Wan Po, 1979; Li Wan Po et al., 1979). The first part of this study was therefore planned to find out whether prior conversion of the 17-ester to the 21-ester was necessary before hydrolysis to the free steroid alcohol by cutaneous esterase.

For elucidating the precise mechanisms involved in the enzymic hydrolysis of the betamethasone esters, initial work was done using a purified esterase. Hog liver carboxylic ester hydrolase was used since a purified cutaneous esterase was not available. Fig. 1 shows the time course for betamethasone-17-valerate and betamethasone when incubated in 20% propylene glycol–Tris buffer at pH 8.14 and 37°C, with and without esterase. First examination of the data (Fig. 1) would suggest that betamethasone-17-valerate is rapidly hydrolyzed to betamethasone by

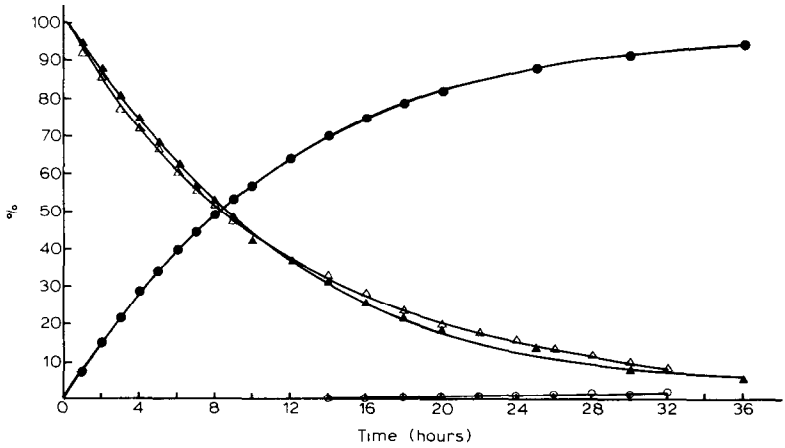

Fig. 1. Time–concentration profiles of betamethasone-17-valerate and betamethasone during the decomposition of the 17-ester (0.1 mg/ml) in 20% propylene glycol–Tris buffer (pH 8.14, 37°C), in the presence and absence of 0.2% esterase. Key: ●, betamethasone formed in presence of esterase; ▲, betamethasone-17-valerate remaining in presence of esterase; ○, betamethasone formed in enzyme-free system; △, betamethasone-17-valerate remaining in enzyme-free system.

the enzyme. At the enzyme concentration used no betamethasone-21-valerate was detected throughout the run. In the control enzyme-free system, on the other hand, the formation of a white precipitate was observed as the reaction proceeded and significant levels of betamethasone-21-valerate were detected in solution. These levels were, however, subject to wide fluctuations during the course of the reaction and no reliable data were obtained for this isomer. This was ascribed to the inconsistent precipitation of the 21-ester and the unreliability of sampling from a suspension. Filtration and dissolution of the precipitate prior to injection into the chromatograph in fact confirmed that the crystals were of the 21-isomer. Closer evaluation of the profiles of betamethasone-17-valerate and of the free steroid alcohol strongly suggests that the 17-valerate was resistant to the enzyme while the 21-ester was highly susceptible. This is shown by the almost identical profiles of betamethasone-17-valerate in both the enzyme-containing and control systems (Fig. 1). A semi-logarithmic plot of the data showed that the disappearance of betamethasone-17-valerate was first-order in both systems.

To overcome the precipitation problem, the initial concentrations of betamethasone-17-valerate and enzyme used were reduced to 0.02 $mg \cdot ml^{-1}$ and 0.004 $ml \cdot ml^{-1}$, respectively, from the previous concentrations of 0.1 $mg \cdot ml^{-1}$ and 0.2 $ml \cdot ml^{-1}$. Fig. 2 shows that under these conditions, the betamethasone-21-valerate remained in solution throughout the experiment and its profile could be plotted without difficulty. Only up to 3% of this isomer was detected in the enzyme-containing system. If the proposal that betamethasone-17-valerate is resistant to the enzyme is valid, then its profiles during the reaction in the presence and absence of the enzyme should be identical and first-order since under these circumstances, the rate equation is that of non-enzymic chemical decomposition. A semi-logarithmic plot of

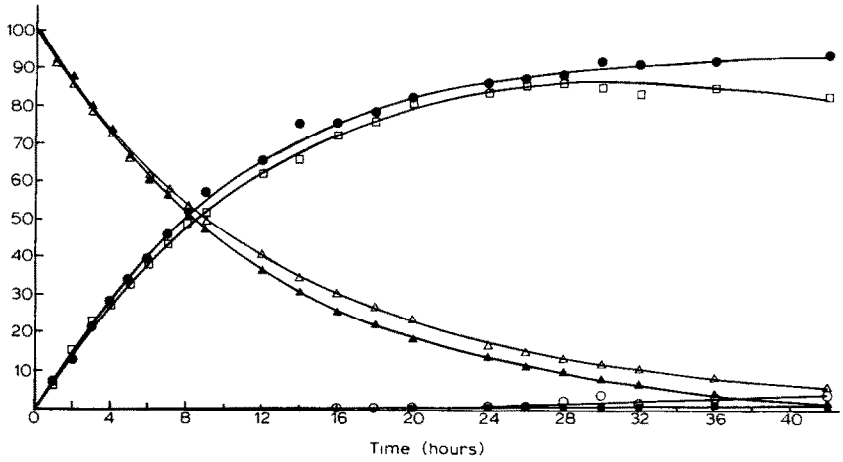

Fig. 2. Time–concentration profiles of betamethasone-17- and -21-valerates and betamethasone during the decomposition of the 17-ester (0.02 mg/ml) in the absence and presence of esterase (0.004%) in 20% propylene glycol–Tris buffer (pH 8.14, 37°C). Key: *in presence of esterase:* ▲, betamethasone-17-valerate; ■ betamethasone-21-valerate; ● betamethasone. *In enzyme-free system:* △, betamethasone-17-valerate; □, betamethasone-21-valerate; ○, betamethasone.

the data showed that the disappearance of the 17-valerate was indeed first-order in both cases (Fig. 3) but the concentration of the 17-ester declined slightly more rapidly in the presence of the enzyme than in the control system. This was a consistent observation and was not due to experimental error. An obvious possible explanation is that the enzyme contributes to the overall disappearance of the 17-ester. A less obvious explanation is suggested by the kinetics of non-enzymic decomposition of betamethasone-17-ester and similar corticosteroid-17-esters (Li Wan Po et al., 1979; Bundgaard and Hansen, 1981). Betamethasone-17-valerate isomerizes rapidly to the 21-ester before slower hydrolysis to the free alcohol. Subsequent work with hydrocortisone-17-butyrate (Yip et al., 1983) has shown that the isomerization is reversible. If this reversibility also applies to betamethasone-17-valerate's isomerization to the 21-ester, then the difference in the profiles of betamethasone-17-valerate in the presence and absence of enzyme can be accounted for by it (Fig. 3). Storage of betamethasone-21-valerate in a system identical to those used in the enzymic studies but initially free of the enzyme and of the 17-valerate confirmed that the reaction was indeed reversible since the 17-ester could be detected by HPLC after storage of the solution. The reverse rate constant was, however, relatively small and the forward to the reverse rate constants for the isomerization was approximately equal to 25. This high ratio explains why despite the reversibility of the reaction, the profiles of the 17-valerate gave good first-order plots. The appropriate rate constants for the reactions as calculated using non-linear regression analysis are shown in Table 1. The data would therefore indicate that in the presence of enzyme, any betamethasone-21-valerate which is formed by non-enzymic decomposition is rapidly hydrolyzed by the enzyme. In the enzymic system the isomerization of the 17-ester is therefore essentially irreversible.

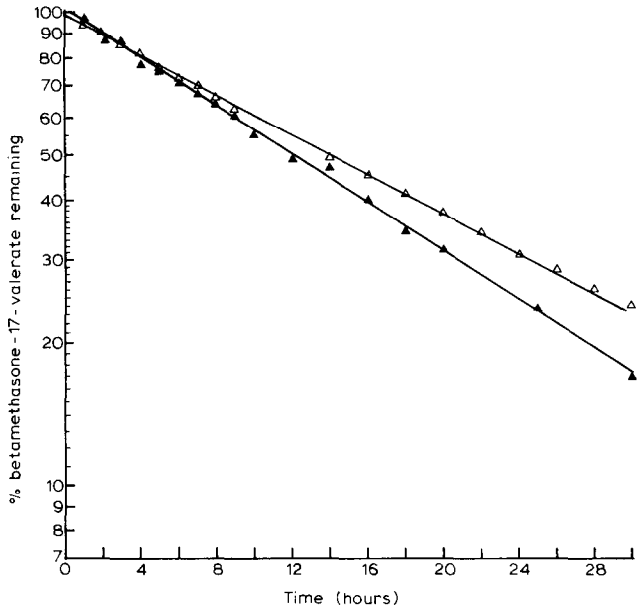

Fig. 3. First-order plot of the decomposition of betamethasone-17-valerate (0.02 mg/ml) in 20% propylene glycol–Tris buffer (pH 8.14, 37°C) in the presence (▲) and absence (△) of 0.2% esterase.

To ensure that the enzyme preparation did not contain any impurities which decomposed the free alcohol, a 0.02 mg · ml^{-1} solution of the latter was incubated in the presence of 0.004 ml · ml^{-1} esterase. No significant decomposition could be

TABLE 1
HYDROLYSIS RATE CONSTANTS OF BETAMETHASONE-17-VALERATE AT pH 8.14 AND 37°C WITH AND WITHOUT HOG LIVER ESTERASE

Initial steroid concentration (%w/v)	Enzymic hydrolysis		Non-enzymic hydrolysis	
	Esterase (%v/v)	Observed rate constant [a] (h^{-1})	Rate constant (h^{-1})	
			Observed [a]	Corrected [c]
0.1	0.2	0.084	–	–
0.1	0.2	0.084	–	–
0.1	–	–	0.079	–
0.02	0.004	0.084	–	–
0.02	–	–	0.076	0.080
0.02	–	–	0.072	0.084
0.06	0.2	0.087	–	–

[a] 20% propylene glycol–Tris buffer.
[b] Rate constant calculated from disappearance of the 17-ester.
[c] Rate constant corrected for reversible isomerization using non-linear regression analysis.

detected up to 40 h under storage conditions identical to those used for the steroid esters.

In studying enzymic decomposition kinetics, the substrate concentration is normally chosen so as to be in excess of the enzyme capacity. The order of reaction for substrate concentration is then zero-order up to the point where the substrate concentration no longer exceeds the enzyme capacity. At this point the order of reaction then changes, usually, to first-order. Linearization of such data with the Lineweaver-Burke type of plots is. of course, well known (Goldstein et al., 1974). A second approach is to monitor the initial reaction rates. This is often resorted to because of frequent divergence between observed and predicted profiles (Dixon and Webb, 1979). In this study, the objectives were to observe the intrinsic enzymic rate constants for the hydrolysis reaction and to elucidate reaction mechanisms. A constant order of reaction throughout would therefore be preferable. It has already been shown that the disappearance of betamethasone-17-valerate follows first-order kinetics in the presence of esterase because this ester is essentially resistant to the enzyme and will only hydrolyze following non-enzymic isomerization to the 21-ester. Although the isomerization is reversible, the forward rate constant is sufficiently fast relative to the reverse rate constant that the data for the 17-ester can be modelled by first-order kinetics even in the absence of enzyme. The data so far indicate that betamethasone-21-valerate is rapidly hydrolyzed by the esterase (Fig. 2). Little of the 21-ester could be detected in the enzyme-containing system while significant amounts were observed in the control system (Fig. 2).

Closer study of the kinetics of enzymic hydrolysis of the 21-ester, however, showed the complexity of the reaction (Fig. 4). A semi-logarithmic plot of the data for the 21-ester showed that the rate of disappearance was again first-order (Fig. 5). This would suggest that the enzyme capacity was not saturated. Monitoring of the decomposition in the presence of varying concentrations of the esterase, however, showed that the rate constant was highly dependent on the esterase concentration (Table 1). Linear regression analysis of the data showed that there was a linear relationship between the enzyme concentration (C, % v/v) and the observed rate constant (k, h^{-1}) in the enzyme concentration range of zero to 0.004 %; a linear relationship which could be expressed by the equation:

$$k = 3291\ C - 0.1 \quad (r = 0.999).$$

To study whether the results of the studies with the hog liver esterase can be extrapolated to skin enzymes, the kinetics of disappearance of betamethasone valerates were followed using homogenates of whole skin from mice. Preliminary studies confirmed that carboxylic acid hydrolase activity was in fact present in the mouse skin. Betamethasone alcohol could be detected in samples incubated with the skin homogenates at concentrations well in excess of those found in an enzyme-free control stored under identical conditions. Hsia et al. (1965) have reported that the co-enzyme NADPH was necessary for restoring the metabolic activity of skin samples obtained from cadavers, to metabolize hydrocortisone. It was therefore felt necessary to check conditions under which the skin homogenates used in the present

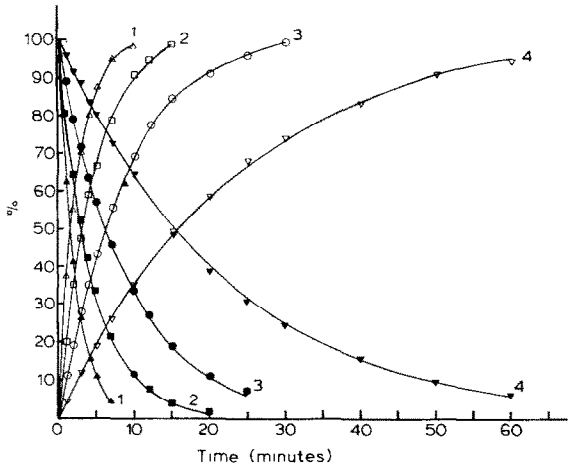

Fig. 4. Enzymic hydrolysis of betamethasone-21-valerate (0.02 mg/ml), by esterase, in 20% propylene glycol–Tris buffer, pH 8.14, 37°C. Key: ▲, ■, ● and ▼ = betamethasone-21-valerate remaining; △, □, ○ and ▽ = betamethasone formed. 1 = with 0.01% esterase; 2 = with 0.004% esterase; 3 = with 0.002% esterase; 4 = with 0.001% esterase.

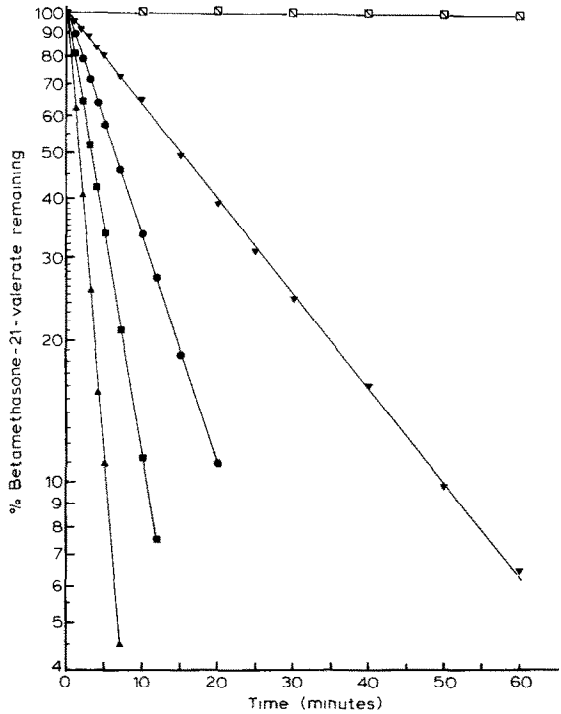

Fig. 5. Enzymic hydrolysis of betamethasone-21-valerate 0.02 mg/ml by esterase, in 20% propylene glycol–Tris buffer, pH 8.14, 37°C. Key: ▲, with 0.01% esterase; ■, with 0.004% esterase; ●, with 0.002% esterase; ▼, with 0.001% esterase; □, control (without esterase).

TABLE 2
EFFECT OF NADPH ON THE ESTERASE ACTIVITY OF MOUSE SKIN HOMOGENATES AGAINST BETAMETHASONE-21-VALERATE (0.06 mg/ml)

Sample	Rate constant of:	
	Decomposition of betamethasone-21-valerate $h^{-1} \times 10^2$	Formation of betamethasone
1	3.4	3.7
2	3.5	3.4
3	3.6	3.9
4	3.2	3.2
5	4.1	3.9

1 = mouse skin homogenates stored for 1 day before use; 2 = mouse skin homogenates stored for 2 days; 3 = mouse skin homogenates stored for 3 days; 4 = mouse skin homogenates stored for 1 day before use and NADPH added; 5 = mouse skin homogenates stored at −15°C before use.

All the samples were stored at −15°C before use and came from the same stock sample of skin homogenates.

study would maintain their enzymic integrity. Under the conditions of this study, using freshly prepared skin homogenates, the same hydrolysis rates were observed for betamethasone-21-valerate with or without NADPH. In stored samples of the homogenates, however, it would appear that the addition of the NADPH helps to stabilize the enzyme (Table 2). When the stored samples were tested, addition of NADPH just prior to use did not reveal any significant activation of hydrolase activity relative to control samples similarly stored (Table 2). Examination of the enzymic activity of the skin homogenates in fact showed no significant drop in activity when stored at − 15°C over a period of 3 days. Despite this, all the studies were, however, carried out within 24 h of sample preparation.

The kinetics of metabolic transformation of betamethasone-17-valerate with the skin homogenates revealed a complication not anticipated in the light of the results from the studies with hog liver esterases. Instead of the marginally lower rate constant of hydrolysis with the control enzyme-free system, the reverse trend was observed. This difference could be duplicated and could not be ascribed to experimental error. A possible explanation was that the ester binds to the components of the skin homogenates and therefore less free betamethasone-17-valerate is available for base-catalyzed non-enzymic degradation. This again adds to the evidence that the 17-ester is essentially resistant to the esterases be they of cutaneous or hepatic origin. The profiles for the 21-ester and alcohol again reveals that the 21-ester is relative to the 17-ester, highly susceptible to the esterases (Fig. 6). Supporting evidence for the binding of all three steroids is provided by filtration studies. If instead of diluting sample aliquots before filtration, as laid down by the assay protocol, these were filtered, and then diluted, significant losses of all three steroids were noted (Table 3).

Fig. 7 shows the enzymic hydrolysis profiles for betamethasone-21-valerate by the mouse skin homogenates when followed over a period of 120 h. Also shown is the

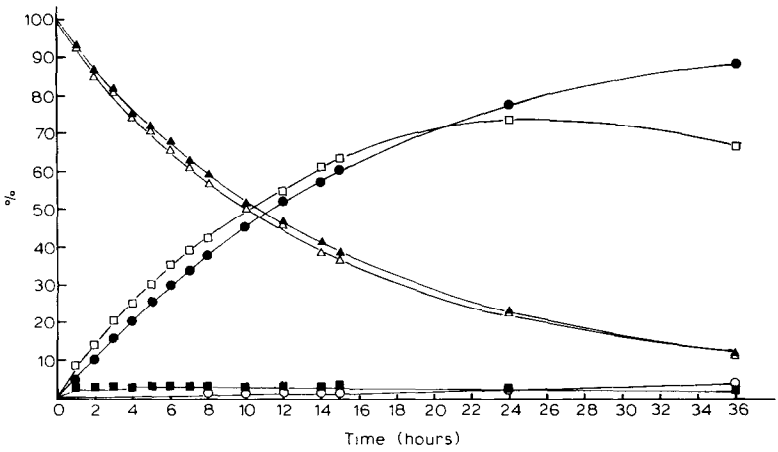

Fig. 6. Biotransformation of betamethasone-17-valerate (0.02 mg/ml) by mouse skin homogenates, in 20% propylene glycol–Tris buffer, pH 7.93, 37°C.

▲, betamethasone-17-valerate remaining } in the presence of mouse
■, betamethasone-21-valerate formed } skin homogenates
●, betamethasone formed }

△, betamethasone-17-valerate remaining }
□, betamethasone-21-valerate formed } in the control
○, betamethasone formed }

profile for betamethasone during the reaction. Although the initial portion of the curve up to about 10 h could be modelled by first-order kinetics, the 21-ester concentrations in the latter stages indicate a decrease in enzymic activity. Beyond 15 h, the enzymic rate of disappearance of betamethasone-21-valerate decreases to equal that of the control enzyme-free system. Various causes may contribute to inhibition of enzymic activity with time. The first is that the enzymes in the skin homogenates are more easily saturable and that what is observed is a transition from saturation or Michaelis–Menten kinetics to first-order kinetics during the reaction.

TABLE 3
EFFECT OF FILTRATION ON STEROID RECOVERY FROM SAMPLES CONTAINING MOUSE SKIN HOMOGENATES, BETAMETHASONE-17-VALERATE (B17) and 21-VALERATE (B21) AND BETAMETHASONE (B)

Sample	% Composition							
	Samples filtered before dilution				Samples diluted before filtration			
	B17	B21	B	Total	B17	B21	B	Total
1	37.6	51.7	5.7	95	39.7	54.7	6	100.4
2		40.3	40.7	81		51.3	50.6	100.9
3		43.3	54.3	97.6		44.9	58.3	103.2
4		34.6	22.1	56.7		43.2	57.3	100.5

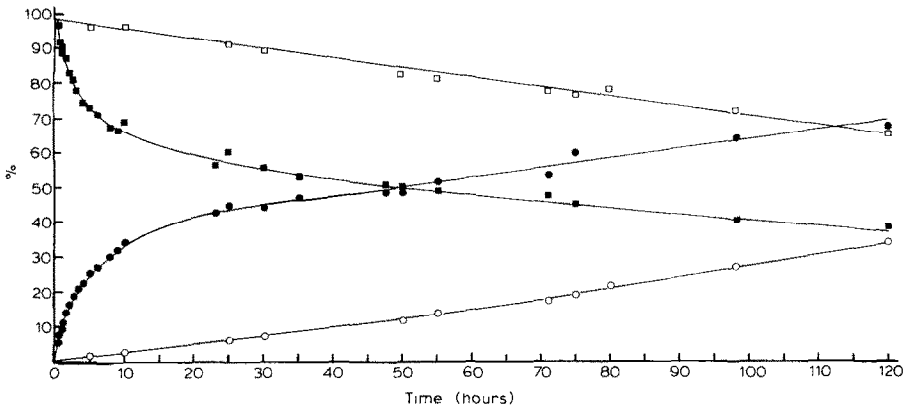

Fig. 7. Biotransformation of betamethasone-21-valerate (0.06 mg/ml) by mouse skin homogenates, in 50% propylene glycol–Krebs buffer, pH 7.5, 37°C. Key: *with mouse skin homogenates:* ■, betamethasone-21-valerate remaining; ●, betamethasone formed. *Control:* □, betamethasone-21-valerate remaining; ○, betamethasone formed.

Kinetic plots with the initial first-order profile show that this was not the case. Other possibilities include inhibition by the reaction products, betamethasone and valeric acid, and enzyme denaturation during the course of the reaction. These possibilities were therefore investigated.

Valeric acid and betamethasone either alone or in combination did not exert any significant effect on the hydrolysis of the 21-valerate as shown in Table 4.

Propylene glycol, however, produced more marked effects particularly upon storage of the enzymic system (Table 5) at 37°C for prolonged periods. It should be noted that in all experiments the final propylene glycol concentration was adjusted

TABLE 4
LACK OF EFFECT OF BETAMETHASONE AND VALERIC ACID ON THE CUTANEOUS ENZYMIC HYDROLYSIS OF BETAMETHASONE-17-VALERATE

Concentration ($mg \cdot ml^{-1}$)		Initial rate constant $h^{-1} \times 10^2$	
Betamethasone	Valeric acid	Disappearance of the 17-ester	Formation of betamethasone
0		8.6	6.63
0.005		8.54	6.65
0.010		7.70	6.70
0.015		7.26	6.40
	0	4.7	5.4
	0.02	5.4	5.9
0.02	0.02	4.93	5.1
0.02	0.02	4.93	4.73

Note that enzyme preparations of different activities were used for the betamethasone on its own experiment and the betamethasone with valeric acid experiment. This explains the different control values.

TABLE 5
EFFECT OF PROPYLENE GLYCOL ON MOUSE SKIN ENZYMIC ACTIVITY AGAINST BETAMETHASONE-17-VALERATE (0.06 mg/ml) AT 37°C IN 50% PROPYLENE GLYCOL–KREBS BUFFER pH 7.54 SYSTEM

Preparation	Initial rate constant ($h^{-1} \times 10^2$)	
	Decomposition of 17-ester	Formation of betamethasone
Enzyme extract only, stored at −15°C for 12 h	8.58	8.78
Enzyme extract stored at −15°C with propylene glycol for 12 h	9.54	9.20
Enzyme extract stored at 37°C with propylene glycol for 5 h	7.98	7.74
Enzyme extract stored at 37°C with propylene glycol for 12 h	4.4	3.84

to be the same, the only variation being during the storage stage. All the data discussed so far therefore suggest that cutaneous esterases are well modelled by liver esterase as far as the hydrolysis of betamethasone-esters are concerned. The results also unequivocably show that the 21-ester is highly susceptible to the esterases while the 17-ester is resistant. In the presence of esterase, the rate-limiting step in the hydrolysis of the 17-ester is its isomerization to the 21-ester. Final confirmation for this is given in Fig. 8 which shows that similar rates of decomposition of the 17-ester was observed in the control and enzyme-containing systems but very different profiles were obtained for the rates of formation of the 21-ester and of the alcohol in the two systems. In the enzymic system, any 21-ester formed is rapidly hydrolyzed so that throughout the run levels remain low.

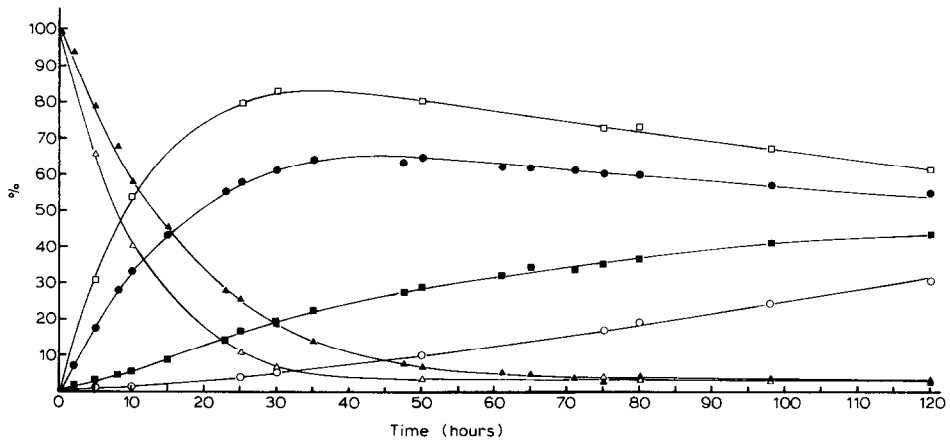

Fig. 8. Biotransformation of betamethasone-17-valerate (0.06 mg/ml) by mouse skin homogenates, in 50% propylene glycol–Krebs buffer, pH 7.54, 37°C. Key: *with mouse skin homogenates:* ▲, betamethasone-17-valerate remaining; ■, betamethasone-21-valerate formed; ●, betamethasone formed. *Control:* △, betamethasone-17-valerate remaining; □, betamethasone-21-valerate formed; ○, betamethasone formed.

Conclusion

The reported data have clearly demonstrated the presence of esterase activity in the skin. They have also shown that skin esterase and liver esterase are very similar in their activity against the steroid esters studied. More importantly, however, the study has shown that the 17-ester is essentially resistant to the esterases while the 21-esters are rapidly hydrolyzed by them. This may explain why 17-steroid esters are more potent topical steroids than the 21-esters. By being resistant to cutaneous esterases, upon application to the skin, they would tend to form better reservoirs in the skin and therefore act for longer periods of time. This may also account for the observation that more potent steroids tend to be more damaging to skin (Somerma et al., 1984).

Acknowledgement

Abstracted in parts from a Ph.D. dissertation presented to the University of Aston by J.W. Cheung.

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